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Expression and Extracellular Release of Human Immunodeficiency Virus Type 1 Gag Precursors by Recombinant Baculovirus-Infected Cells

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The level of synthesis and extracellular release of human immunodeficiency virus type 1 Gag by insect cells was analyzed, using eight different recombinants of *Autographa californica* nuclear polyhedrosis virus harboring various constructs of the *gag* gene, cloned under the polyhedrin promoter. The results obtained suggested that *gag* expression was mainly regulated at the transcriptional level and was not significantly influenced by posttranslational events, e.g., Gag self-assembly, nuclear transport, or extracellular release. Two different forms of Gag were found in the culture medium of recombinant-infected cells. One form consisted of membrane-enveloped, corelike particles released by budding at the plasma membrane; the other of nonparticulate, soluble Gag polyprotein molecules. Both forms coexisted in recombinants expressing Gag with an intact N-terminal myristylation signal, whereas recombinants expressing nonmyristylated Gag released solely the soluble form. This suggested that myristylation of the N terminus was not a prerequisite for efficient extracellular release of Gag by insect cells, which could proceed via two independent but simultaneous mechanisms.

The *gag* gene of the human retrovirus human immunodeficiency virus type 1 (HIV-1) codes for a polyprotein (Gag), which is the precursor of internal structural proteins of the virion (reviewed in references 3 and 5). Posttranslational cleavage of the Gag precursor of 55,000 Da (pr55^{gag}) by the virus-coded protease yields the structural proteins p17MA, p24CA, p9NC, and p6LI (5). All the virion proteins of HIV-1 are potential targets for a class of antiviral agents molecularly designed to bind to structural elements of the virus particle and prevent its assembly or release (21). Spontaneous assembly and budding of HIV-1 corelike particles has been observed in recombinant baculovirus-infected invertebrate cells (6, 16, 22) and recombinant vaccinia virus-infected mammalian cells (8), in contrast to yeast cells (9).

In the present study, we used different forms of HIV-1 Gag polyprotein cloned in baculovirus vectors under the control of the polyhedrin promoter and analyzed the level of expression, intracellular accumulation, and extracellular release of Gag molecules by recombinant baculovirus-infected cells, in correlation with cotranslational and posttranslational modifications, such as N-myristylation, Gag self-assembly, and budding. Eight recombinants expressing different domains of the Gag polyprotein precursor, i.e., p17 plus p24 (pr41), p17 plus p24 plus p9 (pr47), or p17 plus p24 plus p15 (pr55) were studied pairwise, with or without deletion of the N-terminal myristylation (myr) signal. We found that (i) Gag synthesis was mainly regulated at the early steps involving the structure of the polyhedrin promoter and the start codon-flanking sequences, confirming previous observations with other baculovirus-cloned genes (11, 13); (ii) posttranslationally, intracellular assembly of Gag into corelike particles and its transport into the nucleus had no major influence on the final Gag yields; (iii) efficient release of Gag into the extracellular fluid did not significantly affect the overall expression of the *gag* gene; and (iv) the efficiency of extracellular release of Gag polyprotein molecules was apparently not directly related to the presence of an N-myristylation signal or to membrane budding of corelike particles, suggesting the existence of two separate, coexisting mechanisms for Gag extracellular export.

Construction of AcNPV^{gag} recombinants. Four different baculovirus intermediate vectors were used for our constructs. All are pUC9 derivatives (9.8 kbp) containing 7.2 kbp of baculovirus DNA inserted into the *Eco*RI unique site of the pUC9 polylinker. The baculoviral sequence comprises the entire *Autographa californica* nuclear polyhedrosis virus (AcNPV) polyhedrin gene with its promoter elements and flanking sequences from *Galleria mellonella* nuclear polyhedrosis virus. These hybrid baculoviral transfer vectors are referred to as pGmAc. Two of them gave rise to polyhedrin-fused gene products (pGmAc31 and pGmAc533), and two gave rise to nonfused proteins (pGmAc03 and pGmAc115T). Vector pGmAc31 has been deleted between nucleotides +24 and +522, reconstituting a unique *Sma*I site at position +24. The resulting fusion gene product will have the first 8 amino acids of the polyhedrin protein. Vector pGmAc533 has a unique *Cla*I site at nucleotide +173. The fusion protein will have the first 58 amino acids of the polyhedrin, encompassing its karyophilic signal between amino acids 29 and 36 from the N terminus. Vector pGmAc115T has a *Bgl*II linker inserted at a deletion between nucleotides +34 and +407. The polyhedrin start codon has been mutated, with a T replacing the last nucleotide G of the triplet. The resulting constructs preserve the integrity of the entire polyhedrin promoter and the nucleotide sequences adjacent to the former polyhedrin start codon. pGmAc03 has a unique *Sma*I linker inserted at a deletion in the polyhedrin gene sequence spanning from nucleotides −8 to +525 from the polyhedrin A(+1)TG start codon. In both pGmAc115T and pGmAc03, translation of the foreign gene mRNA will start from the first available ATG downstream of the cloning site, resulting in a

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Gag16 M(PDYSYRP^{8})-R^{15}—p17—♦—p24—T^{375}[QIC]

Gag170 M(PDYSYRP^{8})-R^{15}—p17—♦—p24—♦—p9—I^{437}[L]

Gag10 M(PDYSYRP^{8})-R^{15}—p17—♦—p24—♦—p9—♦—p6—Q^{500}

Gag5 M(P—ph—D^{58})-R^{15}—p17—♦—p24—♦—p9—I^{437}[L]

Gag14myr myr-G^{2}—p17—♦—p24—T^{375}[QIC]

Gag13myr myr-G^{2}—p17—♦—p24—♦—p9—I^{437}[L]

Gag12myr myr-G^{2}—p17—♦—p24—♦—p9—♦—p6—Q^{500}

Gag159myr —pro—//—myr-G^{2}—p17—♦—p24—♦—p9—I^{437}[L]

FIG. 1. Polypeptide domains (p17MA, p24CA, p9NC, and p6LI) and N- and C-terminal sequences of recombinant Gag molecules. myr, N-myristylation signal; ph, polyhedrin sequence; pro, polyhedrin promoter. Amino acids starting and ending the cloned Gag sequence are indicated by their single-letter code and their number in the natural Gag sequence (e.g., T^{375} represents threonine of codon 375 in *gag*). Letters in parentheses refer to fused N-terminal polyhedrin sequence, and letters in brackets refer to amino acids foreign to the Gag sequence but specified by cloning linkers or restriction sites.

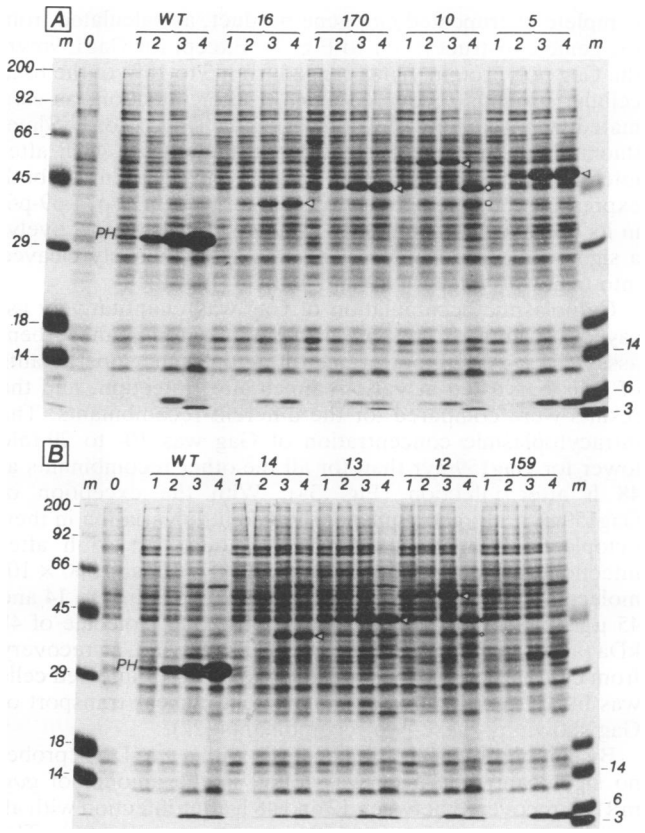

FIG. 2. Polypeptide patterns of baculovirus recombinant-infected *Spodoptera frugiperda* (Sf9) cells. (A) Polyhedrin-fused, N-truncated Gag; (B) Gag with N-myristylation signal. Sf9 cells were infected at a multiplicity of infection of 50 PFU per cell and harvested at 12, 24, 36, and 48 h after infection (lanes 1, 2, 3, and 4, respectively), and whole-cell extracts were analyzed by SDS-PAGE (10). Primary *gag* gene products are indicated by open arrowheads, and proteolytic products from the Gag10 and Gag12myr Gag precursors are indicated by open dots. Lanes: m, prestained molecular mass markers (in kilodaltons) (high and low range; Bethesda Research Laboratories); 0, mock-infected cells. PH, AcNPV-WT polyhedrin. Coomassie blue staining.

nonfusion gene product. Translational initiation at the ATT in this context has been reported (1), but in our constructs, it would result in an abortive chain, out of phase with the rest of the Gag polypeptide sequence. In vivo recombination between baculovirus transfer vector and AcNPV wild-type (WT) DNA was performed in Sf21 cells, and polyhedrin-negative recombinant *gag*-baculovirus clones were isolated by four cycles of plaque purification (25).

The different polypeptide domains and terminal sequences encoded by the various forms of *gag* gene in our eight AcNPV^{gag} recombinant baculoviruses are schematically represented in Fig. 1. The polyhedrin fusion *gag* constructs are referred to as Gag16, -170, -10, and -5, while the nonfusion recombinants are referred to as Gag14myr, -13myr, -12myr, and -159myr, respectively. Gag16, -170, and -10 are the amino-terminally truncated equivalents of Gag14myr, -13myr, and -12myr, respectively. They are a result of the deletion of the N-terminal 14 amino acids of Gag containing the N-myristylation signal, with fusion to the first 8 amino acids of the polyhedrin. Gag16 contains a carboxy-truncated *gag* gene (from *Cla*I to *Sfa*NI at nucleotide 1457 [29]) coding for the polyhedrin-fused p17-MA and p24-CA. Gag170 contains the *Cla*I-*Bgl*II fragment from *gag* corresponding to p17, p24, and p9, deleted of the last 11 amino acids of the p9NC sequence, i.e., 438-WPSYKGRPGNF-448. Gag10 expresses a polyhedrin-fused, full-length *gag* (from *Cla*I to *Bst*XI at position 1916), with its natural TAA stop codon at position 1836. Gag5 encodes the p17, p24, and p9 domains, fused to the N-terminal 58 amino acids from the polyhedrin polypeptide sequence. The Gag polyprotein sequence starts at Arg-15, the preceding residue being common to polyhedrin (Asp-58) and Gag (Asp-14) sequences. Gag14myr, Gag13myr, and Gag12myr were obtained by using pGmAc115T as the intermediate vector. The coding sequence of the N-terminal portion of the Gag polyprotein, between the ATG start codon at nucleotide 336 and the *Pst*I site (nucleotide 961), was synthesized by polymerase chain reaction (23) and controlled by DNA sequencing. The N-terminal myristylation site Met-Gly was thus reconstituted. Recombinant Gag159myr used pGmAc03 as the transfer vector. The blunted *Sac*I-*Hin*cII fragment from pBS-5 was inserted into the pGmAc03 unique *Sma*I site at nucleotide −8 from the A(+1)TG codon of the polyhedrin gene. Gag159myr has the ATG start codon of the *gag* sequence at 110 nucleotides downstream of the natural position of the polyhedrin start codon.

Level of expression of HIV-1 Gag polyprotein in insect cells. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) analysis of whole-cell extracts from AcNPV^{gag} recombinant-infected Sf9 cells gave a crude estimation of the level of Gag polyprotein synthesized at 12, 24, 36, and 48 h after infection. For all recombinants but one, Gag159myr, a major band of cloned protein, absent from the mock-infected or AcNPV-WT-infected cell patterns, was visible after 24 h (Fig. 2). The relative molecular mass of this prominent protein band corresponded to the theoretical mass of the

complete or truncated *gag* gene product, as calculated from the genic construct (refer to Fig. 1). Except for Gag159myr, the Gag polyprotein yields ranged from 5 to 10% of the total cellular proteins recovered at 48 h after infection, as estimated from scanning of Coomassie blue (Fig. 2) or [^{35}S]methionine labeling patterns (data not shown). At 48 h after infection with recombinants Gag10 and Gag12myr, both expressing the complete Gag precursor pr55 (p17-p24-p9-p6) in its N-truncated and non-N-truncated forms, respectively, a significant proportion of Gag was spontaneously cleaved into major products of 49 and 41 kDa (Fig. 2).

Cytoplasmic accumulation of Gag was quantitatively assayed by antigen capture enzyme-linked immunosorbent assay (ELISA) (26) performed on cytoplasmic supernatants of cells harvested at various times after infection, and the results were compared for the different recombinants. The intracytoplasmic concentration of Gag was 10- to 20-fold lower for Gag159myr than for all the other recombinants at 48 h after infection (Fig. 3a). With the exception of Gag159myr, all recombinants showed little variation in their cytoplasmic Gag concentration (Fig. 3a). At 48 h after infection, the values ranged between 180×10^6 and 560×10^6 molecules of Gag per cell (Fig. 3a), corresponding to 14 and 45 μg/10^6 Sf9 cells, respectively, for a Gag molecule of 48 kDa (as for Gag170 and Gag13myr). The lower Gag recovery from cytoplasmic extracts of Gag5- and Gag10-infected cells was likely to be related to the efficient nuclear transport of Gag shown by these two recombinants (22).

By slot-blot hybridization analysis with a *gag* DNA probe, no significant difference was found in the amount of *gag* mRNA recovered between 12 and 48 h after infection with all recombinants, except Gag159myr (data not shown). The proportion of cytoplasmic *gag* mRNA in Gag159myr-infected cells was 7% of the value found in Gag12myr-infected cells at 12 h, 35% at 24 h, and 58% at 48 h, as determined by counting of the hybridized counts. These data confirmed that the level of expression of a foreign gene cloned under the baculovirus polyhedrin gene promoter is greatly dependent on the integrity of the baculovirus nucleotide sequence adjacent to the polyhedrin ATG start codon (11–13, 15). The *gag* gene of Gag159myr was cloned at position −8, whereas all the other *gag* gene constructs either conserved the first polyhedrin ATG as their start codon (fused recombinants Gag16, -170, -10, and -5) or respected the upstream and downstream sequences adjacent to the nonfunctional ATT polyhedrin first codon (nonfused Gag14myr, -13myr, and -12myr). The expression of HIV-1 *gag* would therefore be mainly regulated at the transcriptional level (11, 13, 15), although some control at the translational level could not be excluded (11, 13). However, it has to be considered that the effects observed in our study on baculovirus recombinant-expressed *gag* may not be seen with other viral genes and other host cells. For example, in mammalian cells, *gag* expression is dependent on *rev* coexpression and is regulated by both *cis*- and *trans*-acting mechanisms, involving the RRE sequence in the mRNA and the Rev protein (3, 24).

Intracellular posttranslational events and recombinant *gag* expression. For all the recombinants which respected the polyhedrin promoter structure and the start codon-flanking sequences, additional control could theoretically occur at posttranslational steps and hence modulate the final level of *gag* expression. These late controlling events could depend on the structure and biological properties of the cloned gene product, essentially (i) its cytotoxicity, (ii) its physical exclusion from the cytosolic soluble compartment, (iii) its transport to and accumulation within certain cell compartments, and (iv) its release into the external medium. HIV-1 Gag is apparently not toxic for the insect cell: no extra specific cytopathic effect, other than baculovirus induced, which could provoke some feedback inhibition of protein synthesis was detected with any of our AcNPV^{gag} recombinants. The extent of cellular damage and cytolysis, as

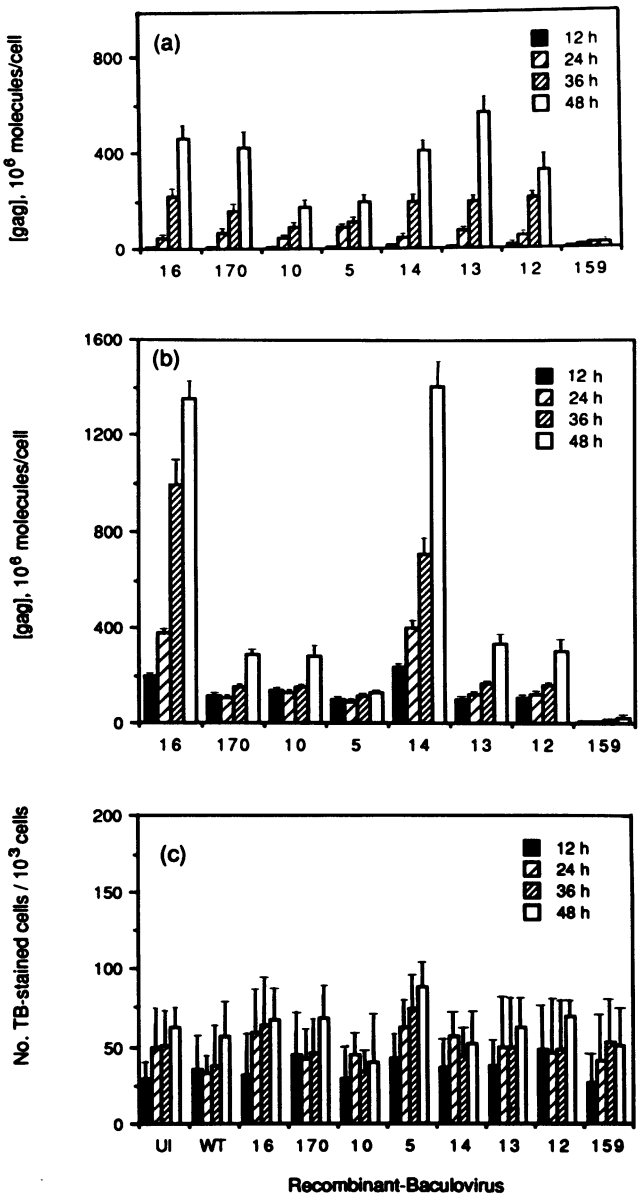

FIG. 3. Quantitative assays of intracytoplasmic (a) and extracellular (b) Gag molecules recovered from uninfected (UI), AcNPV-WT (WT)-infected, or AcNPV^{gag} recombinant-infected cells at different times after infection. Gag concentration, expressed as 10^6 molecules per cell, was determined by antigen capture ELISA, using two anti-HIV-1 p24CA^{gag} monoclonal antibodies and bacterially expressed, chromatographically purified p24CA^{gag} as the standard (26). One microgram of p24CA^{gag} and 1 μg of a 48-kDa Gag precursor correspond to 2.5×10^{13} and 1.2×10^{13} molecules, respectively. Bars indicate standard deviation (n = 4). (c) Cell lysis, determined by the trypan blue (TB) exclusion assay, was quantitatively expressed as the number of TB-labeled cells per 10^3 cells. Bars represent the standard deviation (n = 8).

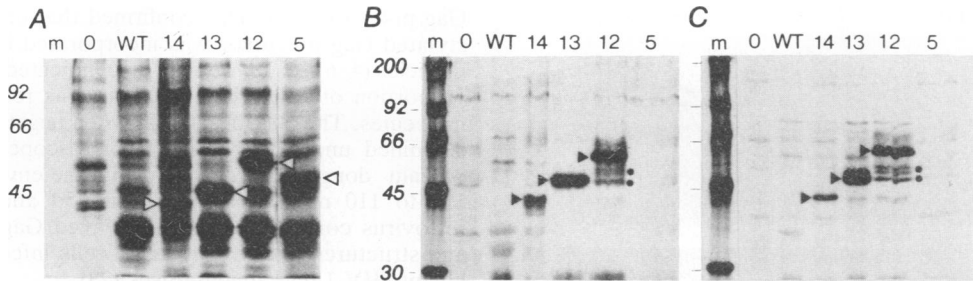

FIG. 4. Polypeptide labeling of Sf9 cells mock infected (lane 0) or infected with AcNPV-WT (lane WT), Gag14myr (lane 14), Gag13myr (lane 13), Gag12myr (lane 12), or Gag5 (lane 5). Cells were labeled with [^{35}S]methionine (A) for 12 h at 16 h after infection (3.7 MBq/ml) or with [^{3}H]myristic acid (B and C) for 20 h at 16 h after infection (6.2 MBq/ml), and whole-cell lysates were analyzed by SDS-PAGE. Gel (shown in panel A) was dried and autoradiographed; gels (shown in panels B and C) were processed for fluorography; the gel in panel C was treated with 1 M hydroxylamine for 18 h prior to fluorographic processing. The efficiency of labeling ranged between 0.3 and 0.6 pmol of [^{3}H]myristyl residue per 100 pmol of Gag. Lane m, ^{14}C-methylated molecular mass markers (in kilodaltons) (Amersham). Recombinant Gag proteins are indicated by arrowheads; cleavage products are indicated by dots.

estimated by a trypan blue exclusion assay, was apparently not significantly different between uninfected cells and cells infected with AcNPV-WT or AcNPV^{gag} recombinants until 48 h after infection (Fig. 3c).

Self-assembly of Gag into intracellular corelike particles with a high efficiency, as shown by Gag170, Gag10, and Gag5 (22), resulted in the exclusion of Gag molecules from the soluble compartments of the cell. In addition, nuclear transport of Gag10, Gag5, and, to a lesser extent, Gag170 (22) prevented the cytoplasmic accumulation of Gag and could enhance their final production. The data showed that the total cellular yields of Gag obtained with Gag170, Gag10, and Gag5 were only slightly higher than those produced by the nonassembling recombinant Gag16 (Fig. 2A), considering the difference in staining intensity between a 41-kDa protein (Gag16) and a 55- or 53-kDa protein band (Gag10 or Gag5). All these suggested that the differences in intracellular pathways of recombinant Gag did not greatly influence the level of *gag* gene expression in Sf9 cells.

N-myristylation and extracellular release of Gag. Release of a cloned protein into the cell culture medium by recombinant-infected cells could also theoretically increase its final production as it diminishes its cytoplasmic concentration. Since N-myristylation represents a membrane-targeting signal for Gag (4, 6), the efficiency of N-myristylation of the different recombinant Gag polyproteins was therefore analyzed by SDS-PAGE and fluorography of [^{3}H]myristic acid-labeled cellular proteins. As shown in Fig. 4, Gag polyproteins expressed by AcNPV^{gag}14myr, -13myr, and -12myr were labeled with [^{3}H]myristic acid, in contrast to polyhedrin-fused Gag. The labeling was hydroxylamine resistant (Fig. 4C), as expected for an N-acylation (14). The intensity of the Gag bands correlated in both [^{3}H]myristic acid- and [^{35}S]-methionine-labeled patterns (Fig. 4A and C). This suggested that the C-terminal deletions of the p6 (Gag13myr) and p15 (Gag14myr) domains had little influence, if any, on the activity of the cellular *N*-myristyl transferase and that N-myristylation of Gag polyprotein occurred cotranslationally in invertebrate cells, as in mammalian cells (27, 30).

Comparison of the quantities of extracellular Gag released by cells infected with the different recombinants, as assayed by antigen capture ELISA (Fig. 3b), showed that they could be grouped into two distinct classes. The first class represented high Gag secreters, which included Gag14myr and its nonmyristylated counterpart Gag16. Both recombinants secreted Gag with a significantly higher efficiency than all the other recombinants which constitute the second class. The observed difference in the amount of extracellular Gag was not simply due to cell lysis since no significant variation in cell viability was detected between low- and high-secreter recombinant-infected cells (Fig. 3c). The cell culture supernatants were also analyzed by SDS-PAGE and immunoblotting, using a pool of monoclonal antibodies directed against the two domains p17MA and p24CA. The immunoblotting patterns of denatured Gag molecules confirmed the ELISA data (not shown) and excluded the hypothesis of a better accessibility of the Gag epitopes in immunocapture ELISA of Gag16 and Gag14myr.

The total (intra- and extracellular) Gag recovery varied between 2,000 × 10^6 molecules per cell for the high secreter Gag14myr and 320 × 10^6 molecules per cell for the low secreter Gag5 at 48 h after infection (Fig. 3), viz., a range of 160 to 25 μg/10^6 Sf9 cells for a Gag molecule of 48 kDa. This was significantly higher than the yields obtained with other expression systems such as vaccinia virus (0.7 μg/10^6 cells [8]), simian virus 40 (10 μg/10^6 cells [24]), and adenovirus (1 to 2 μg/10^6 cells [28]). The amount of extracellular Gag released by the high secreters Gag16 and Gag14myr represented 89 to 71% of the total Gag recovered between 24 and 48 h after infection. For the low-secreter recombinants, the proportion varied between 60 and 40% (Fig. 3). The relatively low difference in the ratio of extracellular to total Gag between high and low secreters suggested that the efficiency of Gag release had no significant effect on the overall level of *gag* gene expression in Sf9 cells.

Nature of extracellular Gag released by insect cells. The properties and structure of extracellular Gag were studied by velocity gradient centrifugation, and the gradient fractions were analyzed by SDS-PAGE, immunoblotting, and electron microscopy. The results were compared for N-truncated and non-N-truncated forms of Gag. Figure 5 shows the results obtained with Gag13myr and its N-truncated equivalent Gag170. Extracellular Gag released by recombinant Gag170 consisted of soluble proteins, with a sedimentation coefficient of 3S to 7S (Fig. 5b). No evidence of particulate structures was found in Gag170-infected cell supernatants by centrifugation analysis or electron microscopy. In the sedimentation pattern of Gag13myr-infected cell culture medium (Fig. 5a), a Gag signal was found at the positions of soluble Gag and of corelike particles (600S; ρ = 1.15). The soluble Gag fractions contained two major bands of 49 and 41 kDa and a discrete doublet band at 25 to 24 kDa, whereas the

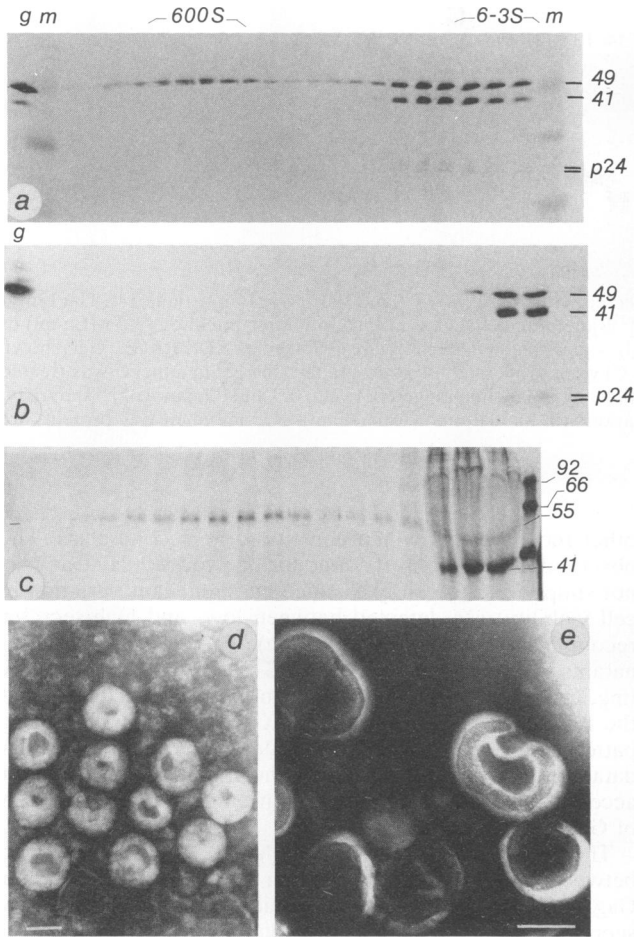

FIG. 5. Velocity gradient centrifugation analysis of extracellular Gag released from cells infected by Gag13myr (a), Gag170 (b), and Gag12myr (c). Aliquots from infected-cell culture media were centrifuged for 90 min at 110,000 × *g* and 4°C in a 25 to 40% sucrose gradient, using virions (800S), deoxycholate nucleoprotein cores (180 to 200S), and groups of nine hexons (60S) and free hexons (13S) of adenovirus type 2 as sedimentation markers (2). Under these conditions, enveloped AcNPV virions sediment at 900 to 1,100S, nucleocapsids at 700 to 900S, and corelike HIV-1 Gag particles at 600S (unpublished data). Fractions were analyzed by SDS-PAGE and immunoblotting with anti-pr55-p24^{gag} antibody (a and b) or by fluorography (c). Lane g, control Gag precursor; lane m, prestained molecular mass markers. Figures on the right are M_r expressed in kilodalton units. Note that the curving of the [^{3}H]myristic acid-labeled bands in the top fractions of the gradient shown in panel c is due to serum albumin present in the culture medium. (d and e) Viruslike particles in the 600S fraction from the gradient shown in panel a observed at different magnifications. Discrete substructures are visible within particles in which the stain has penetrated. Bar represents 100 nm.

particulate Gag fractions consisted of uncleaved Gag precursor of 49 kDa (Fig. 5a). Similar results were obtained with Gag14myr and Gag12myr, which secreted Gag in both particulate and nonparticulate forms, while for Gag16, Gag10, and Gag5, only the soluble form of Gag was released into the culture medium (data not shown). [^{3}H]myristic acid-labeled uncleaved Gag precursor of 55 kDa was found in the 600S fraction of Gag12myr gradient, while the soluble Gag fractions mainly consisted of cleaved N-myristylated Gag pr41 (Fig. 5c). This confirmed that uncleaved N-myristylated Gag precursor was incorporated into budding Gag particles (4, 6, 16, 18, 22), but also indicated that a significant proportion of N-myristylated Gag was released as soluble molecules. The 600S fractions, when negatively stained and examined under the electron microscope, were found to contain doughnut-shaped, membrane-enveloped particles 100 to 110 nm in diameter (Fig. 5d and e), resembling retrovirus cores containing uncleaved Gag precursors (32) and structures released by A549 cells infected with recombinant HIV-1 *gag*-adenoviruses (28).

Conclusions. Recent studies using recombinant HIV-, simian immunodeficiency virus-, or bovine immunodeficiency-like virus-baculoviruses have shown that precursors to p24CA^{gag} can assemble into intracellular 100- to 120-nm corelike particles which are released as pseudovirus particles by budding at the plasma membrane, provided that the precursor molecules are N-myristylated (4, 6, 16, 18, 22). As a consequence of membrane targeting and Gag budding, it was thus expected that N-myristylated forms of Gag would be produced in higher yield than their non-N-myristylated counterparts (31). In fact, a pairwise comparison between recombinants of the high-secreter (Gag16 and Gag14myr) and low-secreter (Gag13myr and Gag170) classes (Fig. 3b) showed that there was no significant difference in the amount of Gag released by cells expressing N-myristylated or non-myristylated forms of Gag. Similarly, intracellular posttranslational events, such as self-assembly of Gag into intracellular corelike particles and their transport to the nucleus, did not greatly influence the level of *gag* gene expression in Sf9 cells, which was found to be mainly regulated at the transcriptional level.

Extracellular Gag released into the culture supernatant of recombinant-infected cells was found to consist of membrane-enveloped, corelike particles and of nonparticulate, soluble proteins. Both forms were released by N-myristylated Gag recombinants Gag14myr, Gag13myr, and Gag12myr, whereas non-N-myristylated Gag expressed by Gag16, Gag170, Gag10, and Gag5 was only released in the soluble form. The high level of extracellular Gag released without membrane budding, as shown by Gag16, suggested that N-myristylation is dispensable for efficient extracellular export of HIV-1 Gag in soluble, nonparticulate form by baculovirus-infected cells. This seemed to be different in mammalian cells (7, 17). Since Gag16 expresses only the p17 and p24 domains, this also suggested that the signal responsible for extracellular release of soluble Gag is located within one or the other domain, between arginine 15 and threonine 375 in the Gag sequence (refer to Fig. 1). This sequence could recognize a plasma membrane Gag receptor, as hypothesized for Rous sarcoma virus (32, 33). The identification of such a sequence in HIV-1 Gag would confirm data obtained with another type of primate retrovirus (Mason-Pfizer monkey virus), implying that a topogenic signal different from the myristylated N terminus could control the transport of Gag to the plasma membrane (19, 20). The finding of significant amounts of extracellular soluble Gag in the culture medium of cells infected with Gag5, which expresses a nucleus-targeted Gag (22), suggested that the polyhedrin karyophilic sequence was not totally dominant over this putative membrane receptor-binding signal.

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